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GENETIC RECOMBINATION IN BACTERIA: A DISCOVERY ACCOUNT

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For the past four decades, bacteria have been favored objects for molecular genetic research. Along with bacteriophages and other plasmids, they have also been instrumental in the contemporary revolution in biotechnology. The importance of bacteria as agents of infectious disease was clearly established by 1876, but this motivated little interest in their fundamental biology until about sixty-five years later. For most of that interval, the genetics of bacteria was a particularly neglected no-man's-land between the disciplines of genetics and of medical bacteriology. Bacteria could not be adopted as models for genetic research until there was some substantiation of the view that they had a genetic system like other organisms. On the contrary, Julian Huxley had once suggested of bacteria that "the entire organism appears to function both as soma and germ plasm and evolution must be a matter of alteration in the reaction system as a whole" (34). Other influential figures like Hinshelwood (32) and Darlington (15) voiced similar views. (Darlington and Huxley, but not Hinshelwood, quickly embraced a more modern perspective when new evidence emerged.)

The question reached closure in 1946 with the demonstration of sexual crossing in the bacterium *Escherichia coli* strain K-12 (66). A brief reminiscence has been published for the fortieth anniversary of that publication (60). That article was joined with some reflections on whether this was a postmature discovery and whether the same inquiry might have been made at a much earlier historical epoch, perhaps promptly after the rediscovery of Mendelism at the turn of the century (103).

The present account concentrates on the scientific milieu and convergent personal histories of Francis J. Ryan (1916–1963) (76, 80), Edward L. Tatum (1909–1975) (59, 61), and myself, Joshua Lederberg (1925–) at Columbia

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University and Yale, culminating in the 1946 publication. If I have any one message to convey, it is an account of my debts: to the individuals who gave so much of themselves as parents, teachers, colleagues, and friends, and to a system that has offered extraordinary nurture to whatever talent and ambition I could bring. That system, the social milieu of science, is under the microscope today, scrutinized for every aberration and pathology. Taken for granted, and thereby overlooked in the presentation of the scientific career to younger people, are its positive aspects of community and of the traditional (and reciprocal!) bonds of teachers and students, not to mention the unique thrills of discovery and the gratification of its application for human benefit.

The pivot of my account is September 1941, when I enrolled as an entering undergraduate at Columbia College in New York City. Although I was born in Montclair, New Jersey, my early education was framed by the New York City public school system. A cadre of devoted and sympathetic teachers went far beyond their duty in encouraging a precocious youngster, despite his taunting them with questions they could not always answer. The culmination was Stuyvesant High School, which specializes in science. Stuyvesant also offered unusual opportunities for practical work in machine shops and analytical laboratories. Most important of all, it attracted a peer group (then unfortunately limited to boys) of the keenest young intellects: for the first time, I had a few intellectual sparring partners. The laboratory opportunities offered at Stuyvesant were augmented by the American Institute Science Laboratory, a forerunner of the Westinghouse Science Talent Search. Instead of offering prizes for the most elegant posters, the AISL offered facilities (in space donated by IBM in the shadow of the Empire State Building) for the conduct of original research, after school hours and on weekends. Here I began to look at the chemical basis of histological fixation and staining: cytochemistry seemed the most challenging point of entry into fundamental biological questions. The New York Public Library was another important element of an efficient and calculated system of Americanization, and of social mobility for first-generation immigrant youth.

My earliest recollections aver an unswerving interest in science, as the means by which man could strive for understanding of his origin, setting, and purpose, and for power to forestall his natural fate of hunger, disease, and death. [Since 1945 the power to destroy has weighed in negative balance on the scientific conscience: we are no longer assured that net human benefit will be achieved as an automatic consequence of the enhancement of knowledge (57, 58). We are not abandoning the enterprise; the global competition, if nothing else, forfends a halt. Weighing the benefit of scientific research has become more complicated.]

The books that engaged me most deeply as a youth, before more advanced texts were accessible, were Eddington and Jeans on physics and inspirational

works like Jaffe's *Crucibles* in chemistry. Wells, Huxley & Wells's encyclopedic *The Science of Life* was the most influential source of my perspective on biology and man's place in the cosmos, seen as evolutionary drama. De Kruif's *Microbe Hunters* turned my entire generation toward a career in medical research. Albert Einstein and Chaim Weizmann were towering culture heroes. The ambitions they inspired were reinforced by a popular culture that idealized the medical scientist with novels and movies like *Arrowsmith, The Magic Bullet, The Life of Louis Pasteur,* and *The Symphony of Six Million.* In a mood born of the Great Depression, however, many of these works painted a bleak picture of the personal life of the scientist: marriage and family were expected to be Baconian "hostages to fortune" (3).

Actual medical textbooks were not so readily available; nevertheless, I was able to read histology, microbiology, and immunology while in high school. Immunology, as then presented, was almost impenetrable to my efforts at orderly, scientific integration. (It took me two decades to realize that the fault was not mine.)

The library book that most impacted my further scientific development was Bodansky's *Introduction to Physiological Chemistry* (7). The copy I received as a Bar Mitzvah present (1938) stands on my bookshelf today, the print almost worn off the pages. This text is medically oriented but covers intermediary metabolism thoroughly, as well as the structure of amino acids and proteins. It also gives an excellent account of Garrod's work on inborn errors of metabolism, a premonition of the founding of biochemical genetics by Beadle & Tatum in 1941 (6). With respect to nucleic acids, nothing is said about their biological function. They are purported to be complexed with protein (by unspecified linkages) to form nucleins. Yeast nucleic acid (also found in plants) contains ribose; thymus nucleic acid contains desoxyribose. Both are tetranucleotides. (All of course quoted from Phoebus Levene.) A second treasured possession was E. B. Wilson's magisterial work, *The Cell in Development and Heredity* (97), a gift for my sixteenth birthday. Published in 1925, this book is probably the most authoritative documentation of pre-1940s biological thought on the cell-biological and biochemical bases of heredity and their relationship to development. Misled by the fluctuating appearances of stained chromosomes at varying stages of compactness, Wilson did attribute the genetic continuity of chromosomes to their oxyphilic (nonnucleic acid) constituents (97a). If he was derailed on this item, we should not overlook Wilson's clarity in seeking explicit mechanistic chemical interpretations in an era that was still shadowed by thoughts of a mystical, life-endowing protoplasm.

With these cardinal inspirations, my entry to Columbia that fall was motivated by a passion to learn how to bring the power of chemical analysis to

the secrets of life. I looked forward to a career in medical research where such advances could be applied to problems like cancer and the malfunctions of the brain.

As it turned out, Columbia was the most fortunate of choices and opportunities. At the time I applied, I doubt if I knew more about Columbia than of its general academic reputation and that Wilson had been on its faculty. The clincher was the award of a tuition scholarship, in the amount of $400 per year, from the Hayden Trust. This, together with commuting from my parental home, made college financially feasible.

My curriculum at Columbia was somewhat topsy-turvy. As soon as a dubious bureaucracy would permit a freshman to do so, I registered in a number of graduate courses in the Department of Zoology. Not until my last senior term did I find the time or maturity to profit from a rounding of my humanistic education at the hands of teachers like Lionel Trilling and James Gutman.

Professor H. Burr Steinbach, who taught the introductory Zoology 1 course, helped arrange a laboratory desk in the histology lab where I could pursue some small research of my own. I had become interested in the cytochemistry of the nucleolus in plant cells the year before, at the AISL. I soon heard of Marcus Rhoades's and Barbara McClintock's cytogenetic research, especially her work on the nucleolar organizer in maize (73a). This introduced me to the uses of genetic analysis in cell biology, and I was soon able to enlist them as helpful counselors.

Professor Franz Schrader's course in cytology introduced me to some of the problems of mitosis (87). I became curious about how the drug colchicine interferes with the mitotic spindle. Herein was my first (albeit trivial) "discovery" in cytotoxicology: an apparent gradient of susceptibility to colchicine down the onion root meristem; but I had no way to answer whether this difference was intrinsic in the cells, or was a transport problem.

This work led to two other starts: (*a*) an effort to induce chromosome aneuploidy in mice by the application of limiting concentrations of colchicine during spermatogenesis, and (*b*) a broader inquiry into the effects of narcotics and other specific inhibitors on the mitotic process. It was easy to disrupt spermatogenesis with colchicine; I saw giant (aneuploid and polyploid) spermatids, but I had no evidence of their successful maturation and functioning in fertilization. It remains, nevertheless, a prototype of potential teratogenesis from anesthetics and other environmental agents. The cytological preparations of colchicine-inhibited mitosis and meiosis were remarked upon by my professors as being strikingly clear for chromosome counts. Had we understood that the karyotype of *Homo sapiens* was problematical, we might have accelerated the recognition (93) that $2n = 46$ (not 48) by over a decade. Salome Waelsch may or may not have approved of my "project," but she was

most encouraging and helpful in providing mice, sometimes to the discomfiture of Professor Schrader in his supervision of the cytology laboratory.

The puzzles of the cytophysiology of mitosis led me to look for courses in cell physiology. However, at that time they were focused on energy metabolism rather than on macromolecular synthesis and fiber assembly. Mendelian genetics seemed to have little relationship to the biology of the cell, presented as it was in the form of combinatorial checkerboards.

I first met Francis Ryan in September of 1942. He had just returned from his postdoctoral fellowship at Stanford University, with E. L. Tatum, to become an instructor in Zoology at Columbia. He brought back the new science of *Neurospora* biochemical genetics and a gift of inspired teaching that was to be a decisive turning point in my own career. I had limited contact with him in formal courses, but by January 1943 I was working in his laboratory assisting in the preparation of media and handling of *Neurospora* cultures. For the first time I was able to observe significant research as it was unfolding and to engage in recurrent discussions with Francis, and with an ever-widening group of graduate students in the department, about *Neurospora*, life, and science. A very cheerful presence in the laboratory was Elizabeth Wilkinson Ryan, who worked (83) alongside Francis through the war years. Lillian Schneider (now Professor Wainright) was Ryan's principal technician after 1943, and also helped enormously to nurture youngsters in the lab and still keep Ryan's research on track.

Ryan had worked with Lester G. Barth at Columbia, in close company with Arthur Pollister and John A. Moore, on the temperature relations of rates of embryological development in frogs. This research was in the tradition of W. J. Crozier and the Chicago school of biophysical physiology. On completing his doctoral dissertation in 1941 (81), Ryan sought a postdoctoral fellowship at Stanford with Douglas Whitaker, with support from the National Research Council, in quest of simpler experimental material, namely *Fucus*. When he arrived at Stanford that fall, Beadle and Tatum had just reported their first findings on biochemical mutants in *Neurospora*, genetically blocked in the biosynthesis of any of a multitude of specific growth factors (5, 6). Ryan implored them to accept him in their lab and was finally accepted, as their first postdoctoral fellow. This was Ryan's own conversion to the power of genetic analysis in the dissection of problems in cellular and general physiology, a zeal he was soon to pass on to me. His work with *Neurospora* began with effects of temperature (and other environmental variables) on growth and on convenient methods of measuring it (84).

Upon his return to Columbia, he extended these methods to the use of *Neurospora* mutants for bioassay of leucine and other nutrients (11, 82, 85). He also began studies on the nutrition, physiology, and chemotherapy of *Clostridium septicum* infection (gas gangrene), which was an important com-

plication of traumatic wounds (83). This work was supported by the Rockefeller Foundation (one more credit to Warren Weaver's historic program in molecular biology) and by the Office of Scientific Research and Development, as part of the mobilization of US science for war-related projects. That support gave Ryan some of the resources that enabled him to take me on as another part-time laboratory helper.

For my own part, I had enlisted in the Navy V-12 college training program upon reaching my seventeenth birthday. The V-12 curriculum for medical officers was designed to compress premedical training to about eighteen months of instruction, and the four-year MD curriculum into three calendar years. My subsequent months at Columbia College were alternated with spells of duty at the US Naval Hospital, St. Albans, Long Island. Here I was assigned to the clinical pathology laboratory, supervised by Commander Harry Zimmerman, a distinguished neuropathologist in his later career at Albert Einstein Medical College. The practical use of my previous training in cytology was the examination of stool specimens for parasite ova and the routine examination of blood smears for malaria among the US Marines returning from the Guadalcanal campaign. This gave me the opportunity to look for the chromosomes of *Plasmodium vivax*. The "chromosomes" were so tiny and the Feulgen staining so faint that it is difficult to insist on the reality of those observations. However, this experience informed me of the sexual stages of the malaria parasite and undoubtedly sensitized me to the possibility of cryptic sexual stages in other microbes (perhaps even bacteria).

In October 1944 I was reassigned to begin my medical course at Columbia College of Physicians and Surgeons (P & S). As a medical student, I continued research on the control of mitosis: namely a search for a hypothetical humoral factor that promoted the rapid regenerative growth of the liver after partial surgical excision (cf. 79). A fellow student, Anthony Iannone, and I had some encouraging responses to parabiosis. However, neither the available assay methods nor our surgical skills and facilities approached what was needed for the task. First-year medical students at P & S were actually discouraged from research, and my intellectual and social environment continued to center on the Morningside Heights campus.

The important biological discovery of 1944 was the identification by Avery, MacLeod & McCarty, at the Rockefeller Institute, of the substance responsible for pneumococcal transformation (1). This phenomenon, which Fred Griffith had stumbled on in 1928 (28), appeared to be the transmission of a gene from one bacterial cell to another; but this interpretation was inevitably obscured by the poor general understanding of bacterial genetics at that time (52). That vagueness was confounded by two outstanding misinterpretations: (*a*) that the transmissible agent was the polysaccharide itself [It is sometimes overlooked that Griffith understood the distinction well enough. Better than

many of his followers, he had at least the germ of a genetic theory: "By S substance I mean that specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate" (28a).] and (*b*) that the agent was a "specific mutagen." For example, Dobzhansky wrote that "we are dealing with authentic cases of induction of specific mutations by specific treatments—a feat which geneticists have vainly tried to accomplish in higher organisms" (19). This formally correct attribution, from a most influential source, obfuscates the idea that the agent is the genetic information.

In retrospect, it is difficult to give proper credit to the logical validity of a large range of alternative interpretations, and to reconstruct the confusions about what was meant by "gene" and "genetic." Recall that until 1951, the only marker observed in transformation was the capsular polysaccharide, the biosynthesis of which was itself subject to many conjectures (e.g. about the role of starter fragments in self-assembly). Avery, undoubtedly somewhat intimidated by Dobzhansky's authority, was reluctant to put his speculations about the genetic significance of transformation in print; his famous letter to his brother surfaced only years later (33, 38, 73, 77). As late as 1948, so distinguished a geneticist as G. W. Beadle still referred to the phenomenon as a "first success in transmuting genes in predetermined ways" (4).

On the other hand, Avery's actual findings were accurately and promptly communicated to Columbia by Dobzhansky (who visited the Rockefeller) and by Alfred Mirsky (of the Rockefeller faculty), who was a close collaborator of Arthur Pollister. The Rockefeller work was the focus of widespread and critical discussion among the faculty and students there. Mirsky was a vocal critic of the chemical identification of the transforming agent. I believe he was quite persuaded that this was an instance of gene transfer, but the more reluctant to concede that the evidence to date settled so important a question as the chemical identity of the gene as pure DNA (versus a complex nucleoprotein). For my own part, the transcendent leap was simply the feasibility of knowing the chemistry of the gene. Whether this was DNA or protein would certainly be clarified in short order, provided the pneumococcal transformation could be securely retained within the conceptual domain of gene transmission. When biologists of that era used terms like *protein*, *nucleic acid*, or *nucleoprotein*, it can hardly be assumed that the words had today's crisp connotations of defined chemical structure. Sleepwalking, we were all groping to discover just what was important about the chemical basis of biological specificity. It was clear to the circle I frequented at Columbia that Avery's work was the most exciting key to that insight.

My own information about the Avery group's work was word of mouth until January 20, 1945 when Harriett Taylor (later Ephrussi-Taylor) lent me her reprint of Avery et al's article (1). At that time she was a PhD candidate,

working at Columbia on the kinetics of growth in yeast; she had already arranged to pursue her postdoctoral studies with Avery at the Rockefeller Institute. My immediate private response to reading the 1944 paper was that the research was "unlimited in its implications. . . . Direct demonstration of the multiplication of transforming factor. . . . Viruses are gene-type compounds [sic]. . . ."

What could be done to incorporate this dramatic finding into the mainstream of biological research; how could one further advance these new hints about the chemistry of the gene? These questions suggested to me the merits of attempting a similar transformation by DNA in *Neurospora*. Not only did this organism have a well-understood life cycle and genetic structure; it also had the advantage of being amenable to selection for rare nutritionally self-sufficient (prototrophic) forms that would facilitate the assay for the transformed cells. And Ryan was working with it in the lab.

In mid-spring 1945, I brought this suggestion to Francis Ryan, who welcomed it as my first research project under his direction. As a brief vacation was looming (to follow rigorous examinations in Anatomy), we agreed to begin in June. However, we soon discovered that the *Neurospora* mutant *leucineless* (allocated to him by Beadle out of the Stanford library) would spontaneously revert to prototrophy. We did not therefore have a reliable assay for the effect of DNA in *Neurospora*. However, the genetic analysis of the reverse-mutation phenomenon resulted in my first scientific publication, with Ryan (86).

Questions about the biological significance of transformation in bacteria would then continue to fester so long as bacteria remained inaccessible to conventional genetic analysis for lack of a sexual stage. But was it true that bacteria were asexual? The standard reply was to mock the fantasies of polymorphisms that were purported exhibitions of sexual union between bacterial cells (60, 103). Most of these surely were attributable to contaminated cultures. Some of the more sophisticated textbooks, and especially Dubos's monograph, *The Bacterial Cell* (20), indeed had footnotes indicating the inconclusive status of claims for sexuality, and pointed out that there had been little genetic testing of this hypothesis. Another important input to this intellectual confrontation was an appreciation of sexuality in yeast, popularized at Columbia via the research work of Sol Spiegelman and Harriett Taylor. Yeast is at least superficially a microbial cousin to bacteria. Gene segregation and recombination in yeast had been demonstrated in 1937 by Winge & Laustsen (98) and then further exploited for physiological genetic analysis by Lindegren (69) and Spiegelman (89). These successes only dramatized the importance of finding a sexual stage, if it existed, in a variety of microbes. If bacteria could be crossed, a new repertoire of biological materials for experimental analysis would be available to physiological genet-

ics and biochemistry. This work might also have important practical applications for vaccine improvement and the understanding of virulence—a latter-day extension of Pasteur's primitive techniques. The compelling motive was to allow the exploitation of DNA transformation in an organism with manifest genetic structure, to further the launching of what is today called "molecular genetics." These were high stakes to justify what was obviously a very long gamble on success (103). Besides having little to lose (I did not need a successful research dissertation for an MD degree), I sensed that no journey on that uncharted ocean would be totally fruitless; even an unsuccessful pursuit of recombination would turn up other phenomena of interest. Such indeed had been my experience with reversion in *Neurospora*, and I have rarely been disappointed since. One cannot be so sanguine today about the opportunity for exploration of new territories, under the pressure for precisely predicted performance that has become pathologically associated with the project system of federal research support.

Some of my notes dated July 8, 1945, articulate, on neighboring pages, hypothetical experiments involving (*a*) a search for mating between the medically important yeastlike fungi, the monilia and then (*b*) the design of experiments to seek genetic recombination in bacteria (by the protocol that later proved to be successful). These notes also coincide, within a few days, with the beginning of my course in medical bacteriology at medical school. They may have been provoked by the repeatedly asserted common wisdom that bacteria were "Schizomycetes," that is, asexual, primitive plants. The basic protocol of these speculative notes entailed the use of a pair of nutritional mutants, say A^+B^- and A^-B^+. If crossing occurred, one could plate out billions of cells in a selective medium if need be: one should be able to find even a single A^+B^+ recombinant. This experimental design was encouraged by Beadle & Coonradt's report of nutritional symbiosis in *Neurospora* heterokaryons (5a). Their speculations [which preceded the finding of recombination in viruses (18, 30)] on the role of heterokaryosis in the evolution of sexual reproduction, offered the bonus that we might find heterokaryosis in bacteria, if not full-blown sexuality. In any event, we would have to be quite attentive to a wide spectrum of possible modes of physiological and genetic complementation.

Dubos's monograph (20) was published and appeared in the Columbia library at a most propitious time, shortly after these speculative ruminations. It furnished an exhaustive and critical review of prior efforts to assess sexuality in bacteria, mainly by morphological and also by genetic methods. Most of these attempts were muddled, but two were more clearheaded (26, 88), albeit with negative findings. But these latter two lacked any selective method for the detection of recombinants. Therefore, the investigators would have overlooked such a process if it occurred in perhaps fewer than one per

thousand cells. All in all, Dubos's analysis substantiated the outlook that the question had never been critically tested.

The principal encouragement to think about genes in bacteria had come from Luria & Delbrück's (1943) experiments on the statistics of mutation in *E. coli* (71). These results supported the view that hereditary adaptive changes, specifically to virus resistance, occurred by spontaneous mutations filtered by selection (i.e. with the bacterial virus). In this respect, at least, there was some evidence that bacteria had "genes," although these experiments do not reach the particulate basis of heredity; they had more to do with a Darwinian than a Mendelian perspective.

One of the principal obscurations to genetic thinking in bacteriology had been the idea that bacteria reacted holistically to environmental insult, that drug or virus resistance was some kind of physiological adaptation that could then become genetically fixed. This anti-Darwinian view was also very much at odds with the gene concept as it had emerged in *Drosophila* studies; but it persuaded many to argue that bacteria did not share the Mendelian organization of their hereditary particles seen in higher organisms. This "last stronghold of Lamarckism" (70) was undoubtedly sustained by sympathy for Lysenko's anti-Mendelism campaign in the USSR. It achieved considerable prestige by being supported by Sir Cyril Hinshelwood, a Nobel-laureate physical chemist and President of the Royal Society, well into the late 1950s. He had the admirable goal of modeling the bacterial cell as a metabolic network, without needing recourse to a specialized store of genetic information. Holistic adaptation, could it but be experimentally verified, would have fitted neatly into his theoretical scheme (32; compare Delbrück, 17).

It is difficult to find a clear instance of a scientific revolution in the history of biology, in the strict sense of a paradigm shift barely coupled to experimental evidence, as enunciated by Kuhn in 1962 (42). The Darwinian revolution comes very close, especially in its application to microbiology. For several decades, the concept of holistic adaptation in bacteria was entertained in the absence of any evidence for it and despite its contradiction to the conceptual framework of population analysis that had emerged for the rest of biology. Today's "DNA revolution" is no less important, but it is related to experimental data more than to such a failure of confrontation.

More explicit encouragement for the possibility of gene recombination in the natural history of bacteria was presented by taxonomic tables of the species or serotypes of *Salmonella* (40). The importance of these bacteria in food poisoning, typhoid fever, and other enteric infections had led to their being studied in a painstaking way to identify antigens helpful in tracking strains through epidemics. As a further consequence, every antigenic strain difference was allowed to attract a novel binomial name, e.g. *Salmonella newport,* which helped commemorate a place—and extend the author's

bibliography. A beneficial side effect of this luxuriant publication was the accessibility of synoptic data that would have been otherwise buried. My reading this literature prompted the speculation that the numerous combinations of somatic and flagellar antigens were generated by some recombinational mechanism.

[As soon as I had my own laboratory and the collaboration of other immunologists and of graduate students, I determined to verify this. That enterprise had the happiest results: the discovery with Norton Zinder of phage-mediated transduction (101), and a series of analyses of the genetics of *Salmonella* antigens with P. R. Edwards, Bruce Stocker, and T. Iino (62, 63, 90). These in turn have furnished exciting models of switches of gene expression based on segmental DNA inversions (10, 12, 35). But all this was to come later.]

The speculation about natural recombination in *Salmonella* also bolstered the idea of looking for it in *E. coli*, as these are very close relatives. For the time being *E. coli* had the advantage of being nonpathogenic (at least for our laboratory strains), and as we shall see, a further advantage was that some nutritional mutants had already been secured in *E. coli*.

Within a few days I set out on my own experiments along these lines—using in the first instance a set of biochemical mutants in bacteria that I laboriously began to accumulate in Ryan's laboratory. None of the well-honed shortcuts we have now (16, 64, 68) were then available, and this was a painstaking process. I was quickly able to get methionine-dependent mutants by selection with sulfonamides, as had been reported by Kohn & Harris (41) (the process is still not really understood). However, the same difficulty as in the *Neurospora* experiments, a spontaneous reversion from A^-B^+ to A^+B^+, had to be accounted for. The strategy would be to use a pair of *double* mutants: $A^-B^-C^+D^+$ and $A^+B^+C^-D^-$. Sexual crossing should still generate $A^+B^+C^+D^+$ prototroph recombinants. These would be unlikely to arise by spontaneous reversions. In theory their occurrence requires the coincidence of two rare events: say $A^- \rightarrow A^+$ and $B^- \rightarrow B^+$. Much effort was devoted to control experiments to verify that double reversions would follow that expectation, and not interfere. The need for double mutants posed a tedious prospect of strain development.

Had a broader range of antibiotics been available, I might already have used selection for multiple drug resistance as an index of crossing (46). However, it was important to use markers closely analogous to those already validated as gene effects in *Neurospora*, namely clear-cut blocks in biosynthesis.

Meanwhile at Stanford Ed Tatum, whose doctoral training at Wisconsin had been in the biochemistry of bacteria, was returning to bacteria as experimental objects, having published two papers on the production of bio-

chemical mutants in *E. coli* (27, 92). During that summer of 1945 Ryan learned that Tatum was about to move from Stanford University to set up a new program in microbiology at Yale. He suggested that rather than ask Tatum merely to share some of his bacterial strains, I should apply to work directly with him and get the benefit of his detailed experience and general wisdom. The war was nearing a victorious conclusion; civilian life and academic schedules might soon be renormalized and make such a visit possible. With Ryan's encouragement, I then wrote Tatum of my research plan (Figure 1) and applied for such an accommodation. Tatum congenially agreed and suggested that I arrive in New Haven in late March 1946, to give him time to set up his laboratory. He had looked into support on my behalf from the Jane Coffin Childs Fund. I had some hint that he may have been formulating similar experimental plans, but these were never elaborated to me. This arrangement suited him by leaving him free to complete the rebuilding of his laboratory, continue his current work in the biochemistry of *Neurospora*, and still follow up the long-shot gamble in looking for bacterial sex.

Once I was at New Haven, my lab efforts were devoted to rechecking the stability of Tatum's existing double-mutant strains, like 58-161 and 679-183 (biotin-methionine and threonine-proline, respectively). Then, additional mutations such as resistance to virus T1 were also incorporated to allow segregation of unselected markers among the prototrophs selected from the mixed cultures on minimal agar medium. It took about six weeks from the time the first serious efforts at crossing were set up in mid-April to establish well-controlled, positive results. By mid-June, Tatum and I felt that the time was ripe to announce them.

A remarkable opportunity was forthcoming at the international Cold Spring Harbor Symposium. This year, it was to be dedicated to genetics of microorganisms, signalling the postwar resumption of major research in a field that had been invigorated by the new discoveries with *Neurospora*, phage, and the role of DNA in the pneumococcus transformation. Tatum was already scheduled to talk about his work on *Neurospora*. Happily, we were also granted a last minute insertion near the end of the program to permit a brief discussion of our new results (65). (I have found no written record of the precise date; the Symposium was scheduled for the week of July 4, 1946.)

The discussion was lively! The most reasoned criticism was Andre Lwoff's concern that the results might be explained by cross-feeding of nutrients between the two strains without their having in fact exchanged genetic information. He was familiar (94) with nutritional symbiosis in *Hemophilus* (72). [I did not think to counterargue that the apparent cross-feeding (94) was actually a genetic exchange. Indeed, *Hemophilus* is now known to accept DNA in a manner analogous to the transformation system in the pneumococcus (25). This counter hypothesis has not, however, been substantiated.]

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19 September 1945

Dr. E. L. Tatum,
Dept. of Botany,
Yale University,
New Haven, Conn.

Dear Sir:

Your recent paper 'X-Ray Induced Mutant Strains of Escherichia Coli' has just come to my attention, and has proven very fascinating. I should be very much obliged to you for reprints of this paper and your preliminary one last summer. I shall take the liberty of writing to you at this length in support of a request that I hope you will entertain.

After doing some work on adaptation (part of which is nearly ready for publication) in Neurospora mutants, it occurred to me that no adequate investigation of a genetic nature had been made to demonstrate the existence or absence of sexual recombination in bacteria. Such things as the distribution of somatic and flagellar antigens in the Salmonella group very strongly suggest that such a process may occur, but no very successful attempt seems to have been made to determine the recombination of bacterial characters. The nutritional mutants described by yourself and Roepke et al. would seem to fill the bill. . . .

I have not yet gone very far in the genetic tests I mentioned (explicitly) on these strains: the methionineless is quite Rough therefore possibly not so satisfactory. I had planned to do essentially what you have accomplished: prepare a double mutant by subjecting the prolineless to the same selective procedure used obtaining methionineless, but that seems unnecessary now for a demonstration that independent (X-ray mutable) genes exist. It has seemed to me, however, that despite the apparent stability of the types I now have, and what is I hope adequate technique to eliminate contamination that it would be highly desirable to have genetically marked strains before any attempt was made to perform the experiment. I should therefore be very much obliged to you for cultures of your biotin double mutant series for the purposes of this investigation. . . .

If an investigation of this sort has already occurred to you, please let me know, as I am sure that you can do a much better job and have better facilities for it than I; on the other hand, if your plans do not include work such as this I should appreciate very much the service I ask of you. . . .

Very sincerely yours,

Joshua Lederberg, A.S. V-12 USNR.

Figure 1 Copy of 1945 letter to E. L. Tatum.

Having taken great pains to control the artifacts from cross-feeding, I felt that the indirect evidence we had gathered, especially the segregation of unselected markers, should be accepted as conclusive, and I spent more argument than necessary on whether more direct proofs need be furnished that the purported recombinants were indeed pure strains. Fortunately, Dr. Max Zelle took me aside after the meeting and most generously offered to advise and assist me in the direct isolation of single cells under the microscope, so as to lay such concerns to rest (100).

The Cold Spring Harbor meetings in 1946 (and again in 1947) were also a marvelous opportunity to benefit from new or renewed introductions to outstanding figures in genetics. Many of the scientists were also extraordinarily supportive human beings, both in thoughtfully listening to the logic of my experiments, and in offering good advice (personal and technical) about how to respond to criticisms. Discussions with figures like Andre Lwoff, Jacques Monod, Guido Pontecorvo, Maclyn McCarty, Seymour Cohen, Bernard Davis, Boris Ephrussi, Raymond Latarjet, Colin Pittendrigh, Curt Stern, C. B. van Niel, Ernst Caspari, J. F. Crow, M. Demerec, Alex Hollaender, Rollin Hotchkiss, Dan Mazia, Howard Newcombe, Elizabeth Russell, Jack Schultz, Wolf Vishniac, M. J. D. White, Evelyn Witkin—and many others—helped to promote lifelong correspondence, and personal and scientific relationships. I remember most vividly the warmth, interest, and friendship offered by Tracy Sonneborn, H. J. Muller, and Salvador E. Luria; later also by Leo Szilard and J. B. S. Haldane, in discussing the work as it unfolded. It is hard to overestimate the importance of these meetings in sustaining the interpersonal network in science.

The most gratifying evidence of the acceptance of these claims by my scientific colleagues was the trickle (later a torrent) of requests for the cultures of *E. coli* K-12 to enable others to repeat the experiments. The first significant confirmatory publications bore the name of Luca Cavalli-Sforza (14), originally from R. A. Fisher's laboratory at Cambridge and later from Milan and Pavia. This prompted the beginning of an extended transatlantic (and later collegial) collaboration with Cavalli-Sforza that was most gratifying both scientifically and personally.

The only studied holdout was Max Delbrück: he quite curtly expressed his disinterest in the phenomenon for the lack of a kinetic analysis. His admonition was immaterial to the claims on the table; it was, however, the kernel of the methodology later used to such good effect by Wollman & Jacob (99) in showing that fertilization was a progressive entry of the chromosome, taking about 100 minutes for consummation. That story has now been well told in Jacob's personal memoir (36a).

By September 1946 I was scheduled to resume my medical studies at P & S, but this was obviously the most unpropitious time to interrupt the exciting

initial progress with crossing in *E. coli*. I was granted another year's leave from P & S and a renewal of my fellowship from the Jane Coffin Childs Fund. The year enabled a consolidation of the preliminary reports and especially the recruitment of many additional genetic markers and the publication of the first linkage map (44). The detailed physical mechanism of crossing was still obscure; it was not, however, mediated by extracellular DNA, for it was quite uninfluenced by the addition of deoxyribonuclease (generously provided by Maclyn McCarty) to the medium. [It would take later discoveries, especially of Hfr (High frequency of recombination) strains by Cavalli-Sforza (13) to open up progress on mechanism.]

A persistent disappointment was the failure of efforts to demonstrate DNA transfer in *E. coli*, which would have completed the paradigmatic aims of the experiment. Boivin & Vendrely had reported such a finding (9), but none of us, Boivin and his collaborators included, was able to reproduce it (R. Tulasne, personal communication; 8), perhaps owing to deterioration of the relevant strains.

Since 1946, *E. coli* K-12 has been the subject of innumerable further investigations, in hundreds if not thousands of laboratories (2). These have substantially revised and enriched our first simple models of the sexual behavior and genetic structure of *E. coli*, though many questions remain open (29, 36a, 37, 53, 55, 56). Many technological applications of gene transfer in *E. coli* have, of course, also emerged. The detailed story of the fructification of the initial discovery is an example of international cooperation and competition that deserves a richer and better informed treatment at some future time.

September 1947 was the next deadline of personal history: I was to return to New York and continue my interrupted medical studies. Ryan also offered me laboratory facilities, and he and Tatum looked hard and partly successfully for some financial support to make all that possible. Meanwhile, Tatum had negotiated with Yale my retroactive registration as a graduate student and had obtained assent from other professors that I had de facto enrolled in a number of their lecture courses and seminars. The work of 1946–1947 became my dissertation, which I had already defended before an international panel of experts. A more serious personal obstacle was obligatory retroactive payment of tuition to Yale University; but the happy result was to qualify for a PhD degree that would, as it turned out, widen my career options. I spent the summer of 1947 at Woods Hole (and the magnificent library of the Marine Biological Laboratory), completing the dissertation. The stacks gave a wonderful opportunity to explore the history of microbiology: how its pioneers had sought to cope with the perplexities of bacterial variability, totally isolated from the intellectual apparatus of modern genetics.

In mid-August, days before the resumption of medical school, I learned from Ed Tatum that the University of Wisconsin had contacted him about an

opening in genetics. In a fashion revolutionary for the time, they were seeking a microbial geneticist! He had recommended my name, and as a Wisconsin graduate his word carried great weight there. I have since learned of the controversy that this proposal evoked. Understandably, the appointment of a 22-year old as an assistant professor warranted close examination. Some referees at Cal Tech were still skeptical about the *E. coli* research: a painstaking review by Ray Owen (at Cal Tech, but recently from Wisconsin) did much to allay concerns in that sphere. Most troubling were allusions about character and race—someone with far stronger suits of tact and polish than mine would have been a more compelling nominee to be among the first Jewish professors in a midwestern college of agriculture. (There have been some happy changes in this country over forty years. We still have many burdens of fairness in meeting the cries for equity from other groups subject to discrimination.) It has been enormously gratifying to have learned in later years of the large effort and integrity of support that were offered by R. A. Brink and M. R. Irwin (at Wisconsin) and by E. B. Sinnott (at Yale). It is a measure of their stature that I was, in the event, offered the position; and when I did come to Madison I was given no inkling of what a struggle I had engendered.

The offer posed the deepest dilemma of my career. I was deeply committed to medical research. Two more years of clinical training (and to be meaningful another two or three of internship and residency) would have reinforced the medical credential, but been a grave (if not total) interruption of research at its most exciting stage. The Wisconsin position was the only one visible for unmitigated support of research in bacterial genetics. That university was furthermore a seat of biochemistry (especially in the Enzyme Institute) and had a long tradition of research in genetics and in microbiology (albeit quite separately up to that point). The Wisconsin Alumni Research Foundation, with income from professors' patents, was a further resource in aiding pioneer research. All this was, however, seated in the College of Agriculture, not Medicine. The medical school at Wisconsin at that time, furthermore, gave little emphasis to research, except for the McArdle Institute for Cancer Research. In short order, I did of course go to Wisconsin, and have never had second thoughts about the wisdom of the choice. The agricultural research context gave me a grounding in practical applications of biotechnology that I have never regretted. I enjoyed a happy collegial association with Brink and Irwin, and shortly thereafter with James F. Crow (whom I had met at the 1947 Cold Spring Harbor symposium) and many other close friends and colleagues, that could be matched at no other time or place. In the long run, however, affiliation with a medical educational and research environment was to be a more compelling vocation. Together with Arthur Kornberg's concurrent move, this was to be the principal attraction of Stanford University, when I was invited to join the new medical school effective February 1959. That

opportunity to return to medically centered activities at Stanford, and later at The Rockefeller University in 1978, has substantiated the advice I had from Tatum in 1947. Nevertheless, among my most cherished honorifics are the MD degrees (honoris causa) that I have received from Tufts and from the University of Turin.

"Contrafactual history" is often derided. Nevertheless, if historical analysis is to go beyond the selection of narrative detail and to assert some theoretical depth, it ought ask "what if?" That is, it should make a plausible cause for "postdicting" alternative outcomes, given different hypothetical inputs. There is, of course, no way to verify such speculations; but unless we indulge in them, how can we speak of learning anything from history?

Without the serendipity of *E. coli* K-12, could sexual genetic recombination have remained undiscovered until this day? As we know in retrospect (47), the choice of *E. coli* strain K-12 was lucky; one in twenty randomly chosen strains of *E. coli* would have given positive results in experiments designed according to our protocols. In particular, strain B, which Delbrück and Demerec had insisted upon as a canonical standard, would have been stubbornly unfruitful. Tatum had acquired K-12 from the routine stock culture collection in Stanford's microbiology department when he sought an *E. coli* strain to use as a source of tryptophanase in work on tryptophane synthesis in *Neurospora* (92). The same strain was then in hand when he set out to make single, and then double, mutants in *E. coli* (91). In 1946 I was very much aware of strain specificities and was speculating about mating types (as in *Neurospora*). I have no way to say how many other strains would have been tried, or in how many combinations, had the June 1946 experiments not worked out so successfully. K-12 has also been the source of the prototypic extrachromosomal elements, F and lambda.

The serendipitous advantages of K-12 notwithstanding, *E. coli* recombination might have been discovered eventually as a byproduct of studies on the infectious transmission of drug resistance, which has become an important practical problem with many pathogenic bacteria. The development of molecular genetics along other paths would have eased the resistance to conjectures about a genetics of bacteria; it would have reduced the incentives to topple the icons; above all it would have vastly multiplied the number of people seeking their own creativity niches (78) in this general area of work. It is hard to imagine that a bacterial conjugation system would not have been discovered at least by the 1950s or 1960s.

Let us stipulate nondiscovery and ask, only partly tongue-in-cheek, how much regret that resynchronization of history would have entailed. Without the *E. coli* system, the optimistic hypothesis is that other paths would have received still more attention. (We cannot be sure that they would have attracted a compensatory interest.) Delbrück and Hershey had already dis-

covered recombination in viruses (18, 30). The discovery of sex in *E. coli* was not a prerequisite for the work of Hershey & Chase (31) on the role of DNA in the virus life cycle, nor that of Watson & Crick (96) on the structure of DNA. Without the distractions of another genetic system like *E. coli*, even more attention might have been paid to the pneumoccoccal transformation and the search for more tractable systems like it.

A significant impediment would have been the lack of detailed genetic maps of the bacterial chromosome; but they might well have been built up piecemeal by other methods. Still more likely would have been less emphasis on bacterial genetics and more on the viruses with their simpler structure. It is conceivable that this would have led to even deeper and more rapid advances at the strictly molecular level, perhaps at the price of a scientific natural history of bacteria, of correlating DNA research with classical genetics, and of some practical advances in biotechnology using bacterial hosts.

Other casualties of the deemphasis of bacteria might have been some aspects of phenomena like plasmids, lysogeny, and lysogenic conversion: the incorporation of viruses into the bacterial chromosome (30a, 43, 50, 54). These concepts have achieved some importance as models for oncogenes. Alternatively, some completely different and even more attractive experimental models, unknown to us at the present time, might have emerged.

Historians should ask about the likely consequences of other counterfacts, stipulated in isolation. They are most provocative if directed at significant discontinuities in the history of science. Besides the further consequences, we can also ask whether such discoveries, perhaps even manyfold, are foreordained in the contemporary milieu (74, 74a, 74b). What if Avery had not pursued the chemistry of the pneumococcus; if Beadle and Tatum had not thought of fungi for biochemical genetics; if Watson and Crick had not pursued the physical chemistry of DNA? Each of these questions has a different genre of answers; on some of them, we might even discover a consensus. These fantasies point to the importance of how attention is focused in the scientific community, a matter as important as, but coupled loosely to, the specific knowledge that is passed on from generation to generation.

Our historical and social understanding does not give us a predictive gauge of the macroeconomics of scientific interest in given fields and the social resources they will attract over periods of time. Every field of enquiry has the latent potential of enormous unforeseeable outcomes. One function of a discovery is to lend credibility to a given avenue of pursuit, and to a new momentum of effort there.

This memoir is dedicated to Francis J. Ryan and Edward L. Tatum. At a time when the public image of scientific fraternity is so problematical, their

lives reflect the survival of norms (74) and behavior exemplifying mutual respect, helpfulness, consideration, and above all a regard for the advancement of knowledge.

Today's popular portrayals of the scientific culture give short shrift to anything but fraud and competition. What contrast to the idealizations by de Kruif and others that inspired my generation! This emphasis may stem in part from the reluctance of scientists to speak out in literary vein, with a few atypical exceptions: outstandingly, June Goodfield in her books and television series (23, 24), which are a renascence of the de Kruif tradition. The competitive stresses on young scientists' behavior today must be acknowledged. The role modeling and critical oversight of their scientific mentors have also warranted celebration (39, 102). These are now complicated by the disappearance of leisure in academic scientific life, the pressures for funding, and academic structures and a project grant system that give too little weight to the nurture and reassurance of the human resources of the scientific enterprise. The often contradictory demands on the scientific personality are ill understood: antitheses such as imagination vs critical rigor; iconoclasm vs respect for established truth; humility and generosity to colleagues vs arrogant audacity to nature; efficient specialization vs broad interest; doing experiments vs reflection; ambition vs sharing of ideas and tools—all these and more must be reconciled within the professional persona, not to mention other dimensions of humanity (21, 75).

I have never encountered the extremities that Jim Watson painted in his self-caricature of ruthless competition in *The Double Helix* (95), which is hardly to argue that they do not exist. Side by side with competition, science offers a frame of personal friendships and institutionalized cooperation that still qualify it as a higher calling. The shared interests of scientists in the pursuit of a universal truth remain among the rare bonds that can transcend bitter personal, national, ethnic, and sectarian rivalries.

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Anyone who knows them, and myself, will recognize how far I have been informed by the sociological insights of Robert K. Merton and Harriet A. Zuckerman. Our retrospection was launched in 1974 during a fellowship year on the history and sociology of science at the Center for Advanced Studies in Behavioral Sciences in Stanford, California. My work in 1946 would not have been possible without the willingness of the Jane Coffin Childs Fund for Medical Research to support a calculated risk, in a way that had few parallels then and has had few since. I am indebted to many colleagues who have furnished invaluable documentary sources. The relevant archival materials will be deposited at the Rockefeller Archive Center, Pocantico Hills, New York, whose staff have also been most helpful throughout this effort. This

article is taken from an autobiographical work in progress, many years short of publication. I appreciate the editors' assistance in selecting those elements most appropriate for the present readership.

Bibliographic Note

A number of items of history, briefly touched upon here, have been reviewed more fully: 45, 48–52, 54–56, 60a, 67.

NOTE ADDED IN PROOF An important encyclopedic overview of *E. coli* genetics has just been announced: Neidhardt, F. C., ed. 1987. Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology*. Vol. 1, 2. Washington, DC: Am. Soc. Microbiol.

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